Caffeine intake, CYPIA2 polymorphism and the risk of recurrent pregnancy loss

Fumihiro Sata1,3, Hideto Yamada2, Kana Suzuki1, Yasuaki Saijo1, Emi H Kato2, Mamoru Morikawa2, Hisanori Minakami2 and Reiko Kishi1

1Department of Public Health and 2Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan
3To whom correspondence should be addressed. E-mail: fsata@med.hokudai.ac.jp

Some case–control studies have demonstrated that caffeine intake and high CYPIA2 activity increase risks of recurrent pregnancy loss (RPL) but the multifactorial effect is obscure. To investigate whether susceptible women who have more caffeine intake are at high risk of RPL, a case–control study of 58 cases with two or more RPL and fertile 147 controls was performed. The association between daily caffeine intake together with CYPIA2*1F (AA versus CA and CC) genotype and RPL was assessed. Without consideration of the genotype, there were no significant differences of the RPL risk in proportion to daily caffeine intake [less than 100 mg (reference); 100–299 mg: odds ratio (OR), 1.29; 95% confidence interval (CI), 0.66–2.50; 300 mg or more: OR, 1.82; 95% CI, 0.72–4.58; P for trend, 0.20]. However, the RPL risk significantly increased only among women who had homozygous CYPIA2*1F alleles with a dosage effect of daily caffeine intake [less than 100 mg (reference); 100–299 mg: OR, 1.94; 95% CI, 0.57–6.66; 300 mg or more: OR, 5.23; 95% CI, 1.05–25.9; P for trend, 0.03]. It was demonstrated for the first time that an increase in caffeine intake deteriorates the fecundity among susceptible women.

Key words: caffeine/CYPIA2/genetic polymorphism/molecular epidemiology/recurrent pregnancy loss

Introduction

About 10–14% of clinically recognized pregnancies end in pregnancy loss in the Japanese population as well as in Caucasians. The etiology of recurrent pregnancy loss (RPL) remains largely unclear (Stirrat, 1990; Parazzini et al., 1991; Cramer and Wise, 2000; Yamada et al., 2001). Epidemiological studies have suggested that the condition might be multifactorial with a possible genetic predisposition and involvement of environmental factors in its pathogenesis (Parazzini et al., 1991; Cramer and Wise, 2000; Yamada et al., 2005).

Some epidemiological studies have suggested that maternal caffeine consumption increases a risk of sporadic pregnancy loss, but the results are inconsistent (Fenster et al., 1991; Infante-Rivard et al., 1993; Mills et al., 1993; Cnattingius et al., 2000; Signorello and McLaughlin, 2004). CYPIA2 is an enzyme primarily responsible for caffeine metabolism, and caffeine clearance rates differ among individuals through genetic propensity and in response to environmental factors (Landi et al., 1999). An increase in caffeine intake has been found to be a risk factor for pregnancy loss among women with high but not low CYPIA2 activity (Signorello et al., 2001). It has been suggested that the homozygous CYPIA2*1F (A/A) genotype, thought to represent a CYPIA2 high inducibility genotype, may either be a direct cause of an increased CYPIA2 activity, or be genetically linked to polymorphisms conferring high inducibility (Sachse et al., 1999).

The aim of this study was to investigate whether the susceptible women who have more caffeine intake are at high risk of RPL.

Materials and methods

This case–control study was performed in the city of Sapporo, Japan, during the years 2003–2004. We conducted the self-administered questionnaire survey to 113 women with a history of RPL and 298 women whose pregnancies ended in live births, who were obstetrically managed in the Hokkaido University Hospital. The questionnaire provided information on medical and obstetric history, maternal age, smoking habits before and during previous pregnancy, and daily caffeine intake during previous pregnancy (i.e. before miscarriages of RPL women and in early pregnancies of fertile women). We estimated caffeine intake according to the frequency questions for coffee (caffeinated and decaffeinated), green tea, black tea, oolong tea and cola as previously described (Nagata et al., 1998). The estimation was based on the assumption that caffeinated coffee contained 40 mg of caffeine per 100 ml, green tea 20 mg/100 ml, black tea 50 mg/100 ml, oolong tea 20 ml/100 ml and cola 30 ml/100 ml. Sixty-four RPL women and 173 fertile women answered the questionnaire and the response rates were 56.6 and 58.1%, respectively. Six RPL women with a uterine conformational abnormality or with couples’ balanced type chromosomal translocation and 26 fertile women who had a history of endometriosis, infertility or who delivered small-for-gestational-age babies were excluded from this study. Thereafter, we studied 58 patients aged 25–43 years with a history of RPL and 147 controls aged 19–44 years. All the patients with RPL and controls were resident in Sapporo and the surrounding areas in Japan; all the patients and the controls were native Japanese. In recent times, the geographical region has had little population immigration by different ethnic groups. The characteristics of the study groups are shown in Table I. RPL was defined as having a history of two or more consecutive pregnancy losses. The primary RPL group compromised 46 women with a history of two or more pregnancy losses but no live birth. The 12 secondary RPL women experienced three or more pregnancy losses after at least one live birth. A total of 53 RPL women experienced all their
one control was unknown.

Passive smoking during pregnancy

Smoking during pregnancy

Caffeine intake (mg/day)

Genotype CYP1A2

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥29</td>
<td>12</td>
<td>52</td>
</tr>
<tr>
<td>30–39</td>
<td>36</td>
<td>87</td>
</tr>
<tr>
<td>≥40</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

Pregnancy loss

2       | 26    | 44.8 |
3       | 17    | 29.3 |
≥4       | 15    | 25.9 |
Primary RPL | 46  | 79.3 |
Secondary RPL | 12  | 20.7 |
<9 weeks | 27    | 46.6 |
9–13 weeks | 26  | 44.8 |
≥14 weeks | 5     | 8.6  |

CYP1A2 genotype

<table>
<thead>
<tr>
<th>Number</th>
<th>%</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>5</td>
<td>8.6</td>
<td>16</td>
</tr>
<tr>
<td>C/A</td>
<td>33</td>
<td>56.9</td>
<td>59</td>
</tr>
<tr>
<td>A/A</td>
<td>20</td>
<td>34.5</td>
<td>72</td>
</tr>
</tbody>
</table>

Caffeine intake (mg/day)

<table>
<thead>
<tr>
<th>Number</th>
<th>%</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–99</td>
<td>21</td>
<td>36.2</td>
<td>65</td>
</tr>
<tr>
<td>100–299</td>
<td>27</td>
<td>46.6</td>
<td>65</td>
</tr>
<tr>
<td>≥300</td>
<td>10</td>
<td>17.2</td>
<td>17</td>
</tr>
</tbody>
</table>

Smoking during pregnancy

Never | 45 | 77.6 | 100 | 68.0 |
Quitter | 8 | 13.8 | 25 | 17.0 |
Continuous | 5 | 8.6 | 22 | 15.0 |

Passive smoking during pregnancy

<table>
<thead>
<tr>
<th>Number</th>
<th>%</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>27</td>
<td>46.6</td>
<td>64</td>
</tr>
<tr>
<td>Yes</td>
<td>31</td>
<td>53.4</td>
<td>82</td>
</tr>
</tbody>
</table>

*One control was unknown.

miscarriages in the first trimester. Twenty-seven of 53 women with RPL experienced all their miscarriages before 9 weeks of gestation, but another 26 women experienced at least one miscarriage between 9 and 13 weeks of gestation, and the other five women experienced at least one miscarriage after 14 weeks of gestation. All women with RPL were subjected to examination by ultrasound and hysterosalpingography for detection of anatomical abnormalities of the genital tract, to chromosome karyotypic analyses of peripheral blood, and to the other RPL screening. The women who had RPL were significantly older than the control subjects (mean 33.8 versus 31.2 years, \( P < 0.01 \)). This study was conducted with all the subjects’ informed consent and approved by the institutional ethical board for human gene and genome studies of Hokkaido University Graduate School of Medicine.

Their blood samples had been obtained at the department of Obstetrics in the Hokkaido University Hospital. Genomic DNA was extracted from the peripheral blood using the QIAamp DNA Blood Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s instructions. Genotyping of CYP1A2*1F polymorphism was determined by either PCR–RFLP (Christiansen et al., 2000; Saijo et al., 2004b) or allelic discrimination using fluorogenic probes and the 5′nuclease (TaqMan) assay as described (Ranade et al., 2001).

Odds ratios (OR) and 95% confidence intervals (CI) were calculated by unconditional logistic regression analysis. Hardy–Weinberg equilibrium analyses were performed to compare observed and expected genotype frequencies using a chi-square test. Furthermore, a caffeine dosage effect \( (P \) for trend) was assessed by modelling a linear effect on the log odds scale for each category of caffeine intake (less than 100 mg/day, 100–299 mg/day and 300 mg/day or more) in a logistic regression model. All analyses were conducted using SPSS software for Windows version 11.0 (SPSS Inc., Chicago, USA).

Results

Without consideration of the genotype, there was a slight tendency to increase the RPL risk in proportion to daily caffeine intake \( [\text{less than } 100 \text{ mg (reference)}; 100–299 \text{ mg: OR, } 1.29; 95\% \text{ CI, } 0.66–2.50; 300 \text{ mg or more: OR, } 1.82; 95\% \text{ CI, } 0.72–4.58; \text{P for trend, 0.20}] \), but with no significance (Table II). The distribution of the CYP1A2 genotype in each group was in Hardy–Weinberg equilibrium. Thirty-three (56.9%) cases were heterozygous, and 20 (34.5%) were homozygous for CYP1A2*1F, compared with 59 (40.1%) and 72 (49.0%) controls.

The RPL risk significantly increased only among women who had homozygous CYP1A2*1F alleles with a dosage effect of daily caffeine intake \( [\text{less than } 100 \text{ mg (reference)}; 100–299 \text{ mg: OR, } 1.94; 95\% \text{ CI, } 0.57–6.66; 300 \text{ mg or more: OR, } 5.23; 95\% \text{ CI, } 1.05–25.9; \text{P for trend, 0.03}] \). However, caffeine intake had no effect on the RPL risk among women who had CYP1A2 CC (*1A*1F) or CA (*1A*1F) genotype (Table II).

Discussion

Recently, many investigations have demonstrated that the maternal genetic polymorphisms related to RPL risk without consideration of the effects of environmental factors (Yamada et al., 2005); these genes included factor V Leiden and prothrombin mutations (Rey

Table II. Associations of maternal caffeine intake during pregnancy with RPL by maternal CYP1A2 genotypes

<table>
<thead>
<tr>
<th>Genotype CYP1A2</th>
<th>Caffeine intake (mg/day)</th>
<th>Cases</th>
<th>Controls</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Total sample</td>
<td>0–99</td>
<td>21</td>
<td>36.2</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>100–299</td>
<td>27</td>
<td>46.6</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>≥300</td>
<td>10</td>
<td>17.2</td>
<td>17</td>
</tr>
<tr>
<td>CYP1A2 CC + CA</td>
<td>0–99</td>
<td>16</td>
<td>42.1</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>100–299</td>
<td>17</td>
<td>44.7</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>≥300</td>
<td>5</td>
<td>13.2</td>
<td>11</td>
</tr>
<tr>
<td>CYP1A2 AA</td>
<td>0–99</td>
<td>5</td>
<td>25.0</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>100–299</td>
<td>10</td>
<td>50.0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>≥300</td>
<td>5</td>
<td>25.0</td>
<td>6</td>
</tr>
</tbody>
</table>

*Adjusted to age and smoking status during pregnancy.
Caffeine, CYP1A2 and the risk of recurrent pregnancy loss

et al., 2003), plasminogen activation inhibitor I and factor XIII (Dossenbach-Glaninger et al., 2003), coagulation factors (Wransby et al., 2000; Plhusch et al., 2001), methylenetetrahydrofolate reductase (Lissak et al., 1999), HLA-G (Aldrich et al., 2001; Pfeiffer et al., 2001), glutathione S-transferase M1 (GSTM1) (Sata et al., 2003a), glutathione S-transferase P1 (GSTP1) (Zusterzeel et al., 2000), interleukin (IL)-1 (Unfried et al., 2001; Wang et al., 2002; Karhukorpi et al., 2003), IL-6 (Saijo et al., 2004a), cytochrome P450 17alpha-hydroxylase/17,20-lyase (CYP17) (Sata et al., 2003b) and endothelial nitric oxide synthase (NOS3) (Temper et al., 2001). However, other genes of xenobiotic-metabolizing enzymes including the CYP1A2 gene by themselves did not significantly affect RPL risk (Saijo et al., 2004b). In the present study, our findings suggest that possession of homozygous CYP1A2*1F alleles may have an increased risk of RPL with a dosage effect of caffeine intake, and provide additional evidence of RPL being a multifactorial disease affected by not only genetic factors but also environmental ones.

Previous studies have assessed the effect of CYP1A2 activity on the risk of sporadic pregnancy loss (Fenster et al., 1998; Signorello et al., 2001). Fenster et al. (1998) performed a case–control study enrolling 73 cases and 141 controls, whereas they found no interaction between CYP1A2 phenotype and caffeine intake in relation to pregnancy loss. On the other hand, Signorello et al. (2001) performed a case–control study enrolling 101 cases and 953 controls and demonstrated that caffeine intake was a risk factor for pregnancy loss among women with high but not low CYP1A2 activity. Both studies were based on the caffeine test using urinary metabolic ratio as an indicator of CYP1A2 phenotype and activity. It was known that the homozygous CYP1A2*1F genotype carried a higher CYP1A2 inducibility than other genotypes (C/C and C/A) (Sachse et al., 1999). A trend towards enhanced activity was observed in pregnant women who had the CYP1A2*1F allele (Nordmark et al., 2002). However, there have been no studies assessing the CYP1A2 genotype and caffeine intake in relation to the RPL risk.

Serum caffeine and paraxanthine, the primary metabolite of caffeine, concentrations were positively related with the caffeine consumption reported by pregnant women, but the serum paraxanthine concentration was more closely related with caffeine consumption than was the serum caffeine concentration, particularly among smokers (Klebanoff et al., 1998). It was reported that serum concentration of paraxanthine was higher in women with RPL than in women who give birth to live infants (Klebanoff et al., 1999). The novel methods for the determination of CYP1A2 activity using paraxanthine/caffeine ratio were proposed to show good predictive performance compared with the conventional method (Doude van Troostwijk et al., 2003). It was reported that homozygous CYP1A2*1F alleles influenced the induction of CYP1A2 activity in vivo (Han et al., 2002). It is possible that paraxanthine may be a key metabolite of caffeine to predispose to RPL because women with CYP1A2*1F alleles could generate paraxanthine more abundantly.

The present study discovered that the caffeine intake during early pregnancy was associated with an increased risk of RPL only among susceptible women. Women who had this high inducible CYP1A2 genotype, homozygous CYP1A2*1F alleles and ingested daily 300 mg or more possessed the highest risk of RPL (OR 5.23). However, caffeine intake had no effects among women who had other genotypes. Thus, we for the first time demonstrated the susceptible genotype with environmental factors such as caffeine intake affecting the human fecundity. We believe that results of this study contain useful information in clinical practice to prevent RPL. It is of interest whether reduction in caffeine consumption early during pregnancy alter pregnancy outcome among women who had a history of pregnancy loss and the homozygous CYP1A2*1F alleles.

In the present study, the response rate of the questionnaire was relatively low, just over 50%. It was possible that the women who took an interest in caffeine consumption or experienced pregnancy loss would answer the questionnaire more frequently. However, in our questionnaire survey we did not inform the women that the frequency of soft drinks questions contained caffeine. There was no significant difference between the response rate of patients with RPL and that of the controls. There was also no significant difference between age or CYP1A2 allelic frequency of the responders and that of the non-responders. Although these two groups, the responders and the non-responders, seemed to be similar according to our limited information, a response bias could not generally be avoidable. Further prospective studies should be performed to confirm our preliminary findings.

Recent investigations outlined that RPL is a multifactorial-polygenic disease (Yamada et al., 2001; Yamada et al., 2005). Molecular epidemiological studies are further needed to unequivocally elucidate the multifactorial effects of both genetic and environmental factors in human fecundity.

Acknowledgements

This work was supported in part by Grants-in-aid for Scientific Research from the Japan Society for the Promotion of Science and the Japan Ministry of Health, Labour and Welfare. We thank Dr T. Kondo, Ms M. Sakuramachi and Ms T. Kunita for their technical assistance.

References


Submitted on February 28, 2005; accepted on April 4, 2005